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Clinorotation Affects Morphology and Ethylene Production in Soybean Seedlings

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The microgravity environment of spaceflight influences growth, morphology and metabolism in etiolated germinating soybean. To determine if clinorotation will similarly impact these processes, we conducted ground-based studies in conjunction with two space experiment opportunities. Soybean (*Glycine max* [L.] Merr.) seeds were planted within BRIC (Biological Research In Canister) canisters and grown for seven days at 20°C under clinorotation (1 rpm) conditions or in a stationary upright mode. Gas samples were taken daily and plants were harvested after seven days for measurement of growth and morphology. Compared to the stationary upright controls, plants exposed to clinorotation exhibited increased root length (125% greater) and fresh weight (42% greater), whereas shoot length and fresh weight decreased by 33% and 16% respectively. Plants grown under clinorotation produced twice as much ethylene as the stationary controls. Seedlings treated with triiodo benzoic acid (TIBA), an auxin tranport inhibitor, under clinorotation produced 50% less ethylene than the untreated control subjected to the same gravity treatment, whereas a treatment with 2,4-D increased ethylene by five-fold in the clinorotated plants. These data suggest that slow clinorotation influences biomass partitioning and ethylene production in etiolated soybean plants.

Key words: Auxin — Clinostat — Ethylene — *Glycine max* L. — Gravity — Partitioning.

Although the microgravity conditions of spaceflight do not appear to affect seed germination (for review see Dutcher et al. 1994, Claassen and Spooner 1994), it can influence subsequent plant growth. Brown et al. (1995) observed that soybean seedlings grown for 8 or 11 days in space had longer roots and higher root fresh weight than the ground controls. Cowles et al. (1984) found decreased growth (measured as plant length, fresh weight and dry weight) for pine, oat and mung bean plants developed in microgravity. Gallegos et al. (1995) noted that sweet clover seedlings were 40% longer relative to the ground controls when grown for 3 days in space. Although these studies suggest that microgravity impacts growth by redirecting whole plant partitioning of assimilates, the mechanism is not clear.

The slowly rotating horizontal clinostat is a ground-based apparatus used to create a gravity vector-averaged environment and is sometimes useful to predict potential spaceflight effects on biological specimens (Albrecht-Buehler 1992). Several reports describe the effects of clinorotation on plant physiology such as cytoskeletal function (Hilaire et al. 1995a), protein expression (Piastuch and Brown 1995), carbohydrate metabolism (Brown and Piastuch 1994, Obenland and Brown 1994), calcium distribution (Hilaire et al. 1995b) and the cell cycle (Legué et al. 1992).

Ethylene, $(CH_2)_2$, is a flammable volatile plant hormone which diffuses in the gas phase through the intercellular spaces and outside the tissue (Jackson 1991). Under non-stress conditions, ethylene release occurs during different developmental stages of the plant such as fruit ripening and senescence (McAfee and Morgan 1971). There are many examples of increased ethylene production following stresses induced by abiotic or biotic agents. For example, plants exposed to supraoptimal temperatures (Field 1981) or flooding (Kawase 1976) produced increased amounts of ethylene. At the cellular level, ethylene has been shown to affect microtubule orientation (Lang et al. 1982), to increase cell wall thickness (Freytag et al. 1977), and to increase respiration and concentration of fructose 2,6-bisphosphate (Stitt et al. 1986). Several authors also reported an increase in ethylene production by plants exposed to clinorotation (Leather et al. 1972, Hensel and Iversen 1980, Driss-Ecole et al. 1994). Brown et al. (1995) extended these experiments to the microgravity environment and observed a two-fold increase in ethylene production in soybean seedlings grown in space relative to the ground controls.

The objectives of the present study were to determine (1) the influence of clinorotation on the growth, morphology and ethylene evolution of soybean seedlings and (2) if the addition of synthetic auxin or auxin transport inhibitors would affect the concentration of head space ethylene due to clinorotation. This study revealed an effect of clinorotation on soybean seedling morphology associated with an increase in ethylene production.

Abbreviations: BRIC, Biological Research In Canister; FAA, formaldehyde acetic acid; STS, Space Transportation System; TIBA, triiodo benzoic acid.

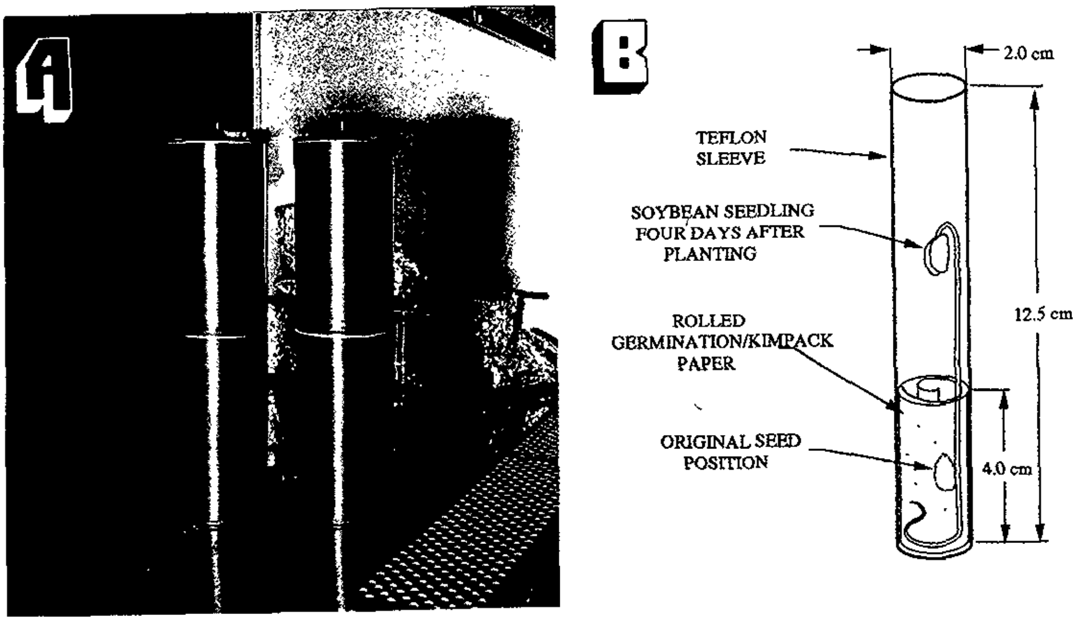


Fig. 1 (A) BRIC (Biological Research in Canister) ground support equipment hardware and (B) Teflon sleeve, germination paper seed germination apparatus.

Materials and Methods

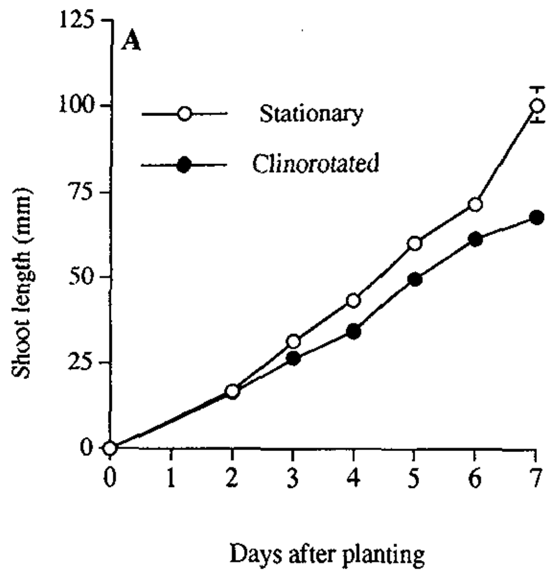
Soybean (*Glycine max* L. [Merr] cv. McCall) seeds were germinated and grown under conditions of slow clinorotation (1 rpm) or maintained stationary for 7 days in BRIC (Biological Research in Canister) ground support hardware (Fig. 1A) at 20°C. These canisters consist of two independent compartments, each of which is 8.2 cm (diameter) by 31.2 cm (height). Each compartment contains 4 light-tight, passive pressure relief vents (1.5 mm in diameter) which were used as gas sampling ports. Individual seeds were rolled in germination paper, each roll was inserted into a Teflon tube (Fig. 1B) and each canister compartment contained 13 of such tubes. To initiate the experiment, 2.5 ml of sterile water, 10^{-4} M TIBA or 10^{-5} M 2,4-D was added to each roll. Duplicates gas samples (0.5 ml) were withdrawn daily from the head space of each canister compartment using gas-tight syringes. Analyses of the amounts of ethylene and CO_2 were performed immediately after sampling. Each gas sample was injected in a gas chromatograph equipped with a thermal detector for CO_2 (Hewlett Packard gas chromatograph 5880) or a photoionization detector for ethylene (Photovac gas chromatograph 10S70). The detector response was standardized against injections of known quantities of CO_2 or ethylene prepared by serial dilutions.

After the daily gas sampling, plants were removed from independent canisters and the shoots and roots were measured and weighed. The tissue was fixed in FAA and processed for light microscopy. Cross sectional areas of the shoot and root were analyzed by computer using the NIH Image 931220 software. Shoot sections were taken midway between the root/shoot interface and the hypocotyl hook. Root sections were taken midway between the root/shoot interface and the root tip.

Results

Soybean seedlings grown for 7 days on the horizontal clinostat at 1 rpm showed a decrease in shoot length by 33% (Fig. 2A) and a doubling of root length compared to the stationary controls (Fig. 2B). This difference in shoot and root length became evident by 4 to 5 days after planting. Shoots from the clinorotated plants were characterized by a decrease in fresh weight whereas roots from these plants had a greater fresh weight relative to the stationary controls (Table 1). Shoots from the clinorotated plants had a larger diameter relative to the stationary control whereas roots from the clinorotated plants had a smaller cross sectional area compared to the controls (Table 2).

Daily gas sampling from the canister revealed an increase in ethylene production through time in the stationary upright controls. By day 7 after planting, however, soybean seedlings grown under clinorotation conditions produced twice as much ethylene as the stationary control (Fig. 3A). The CO_2 concentration also increased through the plant development but no significant differences were found between the clinorotation treatment and the stationary control (Fig. 3B). When clinorotated seedlings were grown in the presence of an auxin transport blocker (TIBA), the amount of ethylene produced was two-fold lower starting at day 5 compared to the amount produced by clinorotated plants grown in water (Fig. 4A). The level of CO_2 produced by the TIBA treated plants was always higher than the control until day 7 where they were identical (Fig. 4B). On the other hand, treatment of the seedlings



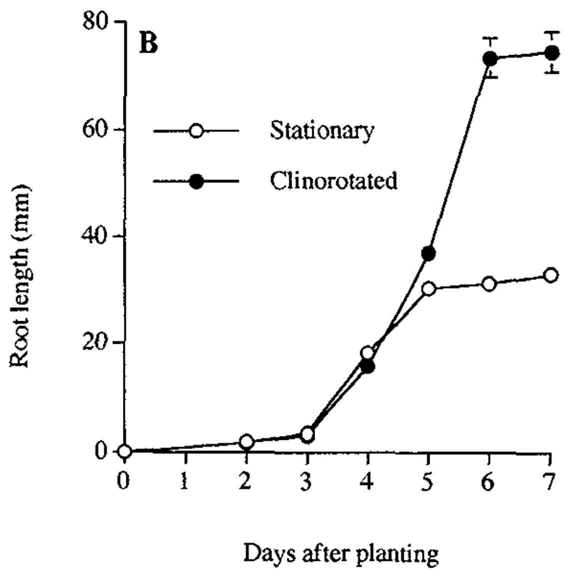


Fig. 2 Soybean seedling growth in canisters under two conditions: upright stationary and horizontal clinorotation (1 rpm). Each symbol represents the mean value (n=26-50±SE). Notice the decrease in shoot length (A) and the increase in root length resulting from clinorotation (B).

with 2,4-D during clinorotation resulted in a five-fold increase in ethylene production relative to the plants grown in water at day 5 after planting (Fig. 4A) and thereafter the level starts to decrease to return to the same level as the control in water only. The seedlings treated with 2,4-D produced more CO_2 relative to the control until day 7 where both levels were very close (Fig. 4B).

Table 1 Fresh weight of shoot and root tissue of seven-day-old etiolated soybean plants grown in stationary, upright position or exposed to clinorotation (1 rpm)

	Fresh weight (mg)		% Difference
	Stationary	Clinorotation	
Shoot	584 (24)	494 (24)*	−16%
Root	87 (8)	124 (8)*	+42%

Values represent the mean (n=26) and the standard error is shown in parentheses.
* *Statistically different at the 5% level of significance.*

Discussion

Clinorotation increases root growth relative to shoot growth in etiolated soybean seedlings, consistent with similar observations by Hoson et al. (1992) with azuki bean using a three-dimensional clinostat. These particular morphological changes have been observed previously in space-grown plants (Brown et al. 1995, Gallegos et al. 1995). Huber (1983) showed that starch stored in leaves during the day was degraded at night and preferentially translocated (as sucrose) to the root for growth. It has been shown that starch concentration in the cotyledons of clinorotated soybean seedlings is lower, perhaps indicative of diminished starch synthetic capability (Brown and Piastuch 1994). This suggests that the enhanced root growth under clinorotation may be related to the lower starch concentration due to enhanced carbon translocation from the cotyledons. Microscopical analysis showed that the effects of clinorotation on growth are associated with an increase in the diameter of the shoot and a decrease for the root.

Table 2 Cross sectional areas of stem and root tissue[a] of seven-day-old etiolated soybean plants grown in stationary, upright position or exposed to clinorotation (1 rpm)

	Area (mm^2)		% Difference
	Stationary	Clinorotation	
Shoot	5.0 (0.3)	6.7 (0.5)*	+34%
Root	1.6 (0.2)	0.5 (0.1)*	−69%

Values represent the mean (n=10) and the standard error is shown in parentheses.
[a] Shoot sections were taken midway between the root/shoot interface and the hypocotyl hook and root sections were taken midway between the root/shoot interface and the root tip.
* Statistically different at the 5% level of significance.

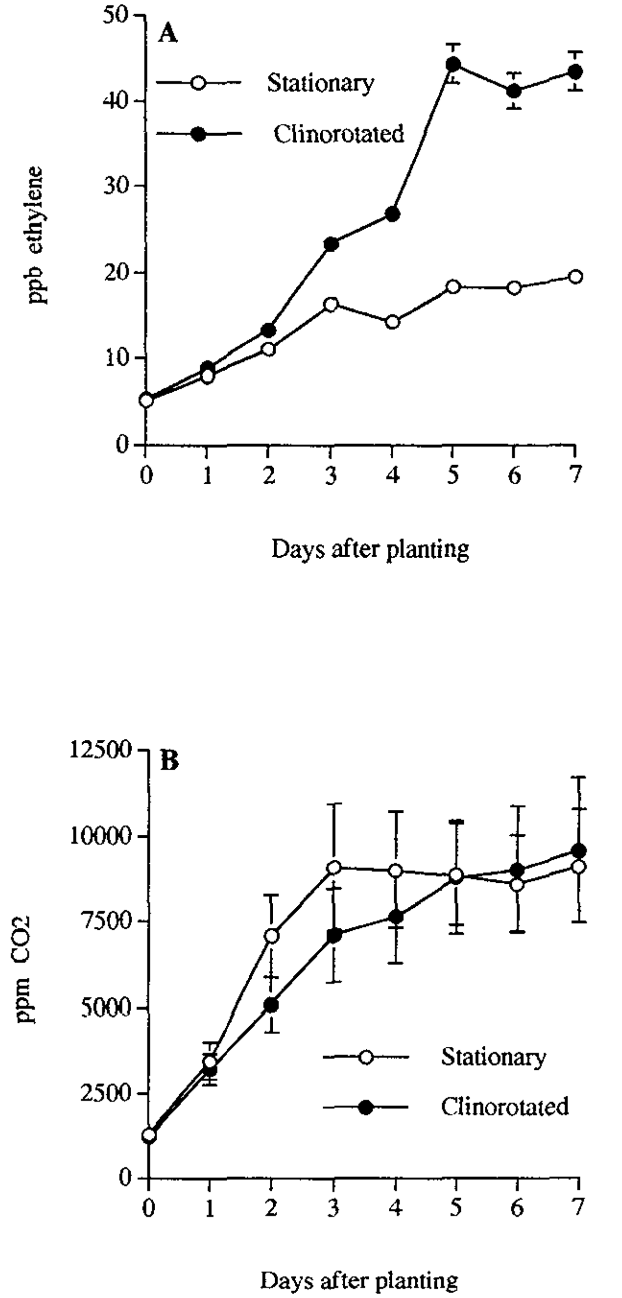


Fig. 3 (A) Ethylene and (B) CO_2 concentrations in the BRIC headspace with soybean seedlings grown under stationary conditions or under horizontal clinorotation (1 rpm). Each symbol represents the mean value (n=15–16±SE).

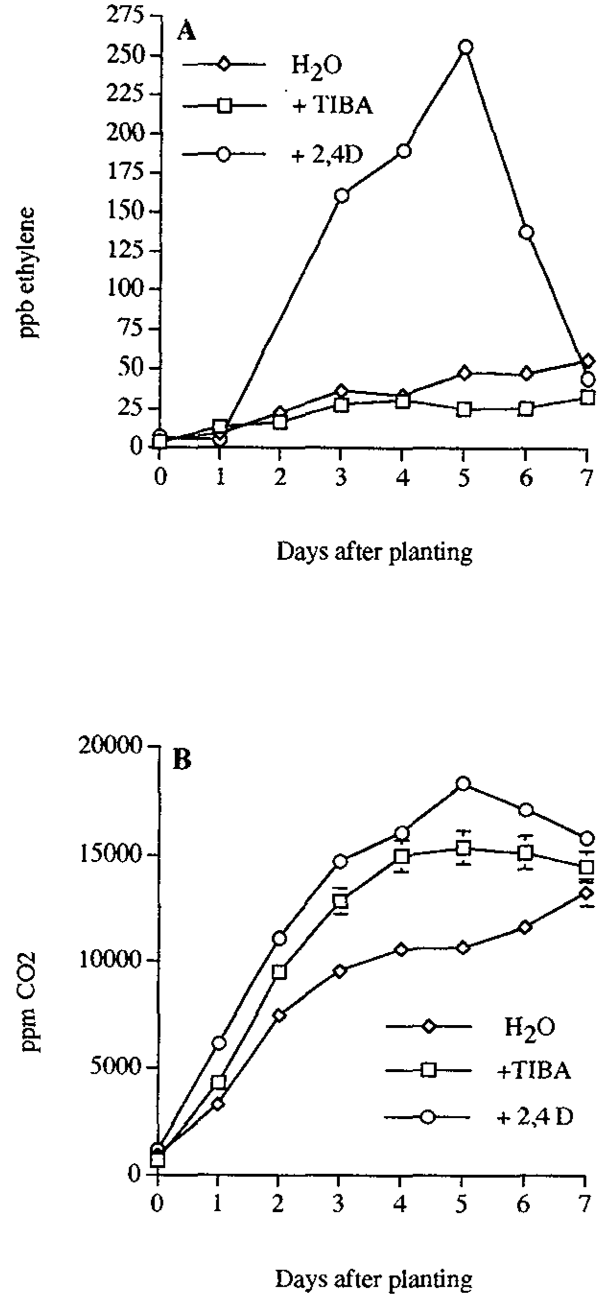


Fig. 4 (A) Ethylene (B) CO_2 concentrations in the BRIC headspace with soybean seedlings grown under horizontal clinorotation (1 prm) in the presence of water, 10^{-4} M TIBA or 10^{-5} M 2,4D. Each symbol represents the mean value (n=15–16± SE).

Gas measurements revealed an increase in ethylene production during clinorotation. Taken together, these data suggest that the increase in ethylene due to clinorotation may affect root and shoot tissue morphology. Leather et al. (1972) reported that 8-week-old tomato plants produced twice as much ethylene after two hours of clinorotation at 0.2 rpm relative to the controls. This rise in ethylene concentration was associated with leaf epinasty. Hensel and Iversen (1980) observed that 24-h-old cress seedlings produced at least three times more ethylene than the stationary controls after five hours of clinorotation at 2 rpm. The same authors also found that the root length of the clinorotated seedlings was 81.4% of that of the controls.

Both of these experiments described a rise in ethylene within hours after clinorotation whereas we observed a similar increase after seven days of growth and clinorotation, indicating that the clinorotation-induced ethylene production is not a short-term transient effect. This is corroborated by a more recent study from Driss-Ecole et al. (1994) who grew *Veronica arvensis* plants for 45 days on a clinostat rotating at 1 rpm and found that ethylene production was higher in the clinorotated plants relative to the rotating vertical controls. However, when ethylene concentration was expressed relative to plant biomass no differences were found. These authors observed that the hypocotyl and root dry weight from the clinorotated samples was increased by 40% relative to the controls, explained by an increase in secondary root production. In our experiment, 7-day-old clinorotated soybean seedlings had longer main roots than the stationary controls but very little secondary roots. If the experiment would have continued for a longer period of time, it is possible that enhanced secondary root growth would have occured.

Root elongation has been shown to be promoted (Dedolph et al. 1965, Smith and Robertson 1971, Koning and Jackson 1979, Nakayama and Ota 1980) or inhibited by ethylene (Chadwick and Burg 1967, Rauser and Horton 1975, Whalen and Feldman 1988), while other reports suggest an ethylene-induced decrease in shoot growth (Warner and Leopold 1971, Goeschl and Kays 1975, Nee et al. 1978). Therefore, it is possible that the alteration in root/shoot ratios which we observed upon clinorotation are due in part to an ethylene buildup. We observed that ethylene production was significantly higher than the stationary control at day 3 after planting. Since at that age the seedlings are still very short (about 30 mm) and therefore are still firmly maintained within the germination paper, it is likely that this increase in ethylene production is due to a disturbance in gravity perception produced by clinorotation rather than to mechanical stress, such as the contact of the seedlings with the teflon sleeve. In agreement with our results is the report from Salisbury and Wheeler (1981) who showed that leaf epinasty observed in clinorotated plants was due to ethylene production and that epinasty was caused by disturbances in the gravity perception mechanism rather than leaf flopping.

Previous studies reported that high ethylene concentration causes seedling swelling (Bertell et al. 1990, Camp and Wicklife 1981) by affecting microtubule and cellulose microfibril orientation (Eisinger 1983), therefore altering seedling growth (Smith and Robertson 1971). Clinorotation did not alter CO_2 concentration significantly compared to the stationary controls. Previous studies from spaceflight experiments also report an increase in ethylene production in microgravity but no changes in CO_2 (Brown et al. 1995, Gallegos et al. 1995) suggesting that both horizontal slow clinorotation and microgravity represent stressful environments for the plants. However, in both of these space experiments gas samples were taken after the seedlings were back to Earth, therefore the increase in ethylene production may be due to the stress produced by shuttle re-entry in the atmosphere and/or to re-exposure to Earth unit gravity and not to microgravity. Caution must be taken in interpretating the results because even if an increase in ethylene production was observed in clinorotated samples and in some space flight samples (Brown et al. 1995, Gallegos et al. 1995), it does not necessarily imply that the cellular mechanism leading to this result is identical. In the case of clinorotation, the constant movement of the amyloplasts within the columella cells (Hilaire et al. 1995a) may constitute a cellular mechanical stress (as opposed of a physical contact which would be an external mechanical stress) whereas in the case of space environment it would be the reduced gravity. In both conditions, the stress is constant as well as the increase in ethylene production. In addition, it is important to note that ethylene has been proposed to be required in stem gravitropism (Wheeler and Salisbury 1980). Therefore, during gravistimulation the ethylene production may be enhanced in a transient manner and correlated with the generation of a curvature, possibly due to the rapid movement of the amyloplasts from one region of the columella cells to the new distal pole.

When treated with an auxin transport inhibitor (TIBA) or a synthetic auxin analogue (2,4-D) during clinorotation, soybean seedlings produced more CO_2 than the control and reached a plateau faster. In addition, the TIBA treatment during clinorotation reduced the production of ethylene by the plants whereas addition of 2,4-D increased ethylene concentration considerably. Exogenously added 2,4-D is subsequently degraded (Kang et al. 1971) and presumably is no longer effective, which could explain the drop in ethylene concentration observed after day 5.

Previous studies have explored the relationship between auxin and ethylene production. For example, Romano et al. (1993) reported that overproduction of auxin in transgenic plants resulted in the overproduction of ethylene. In addition, Yoshii and Imaseki (1981) showed that auxin promotes ethylene production by inducing the synthesis of ACC synthase, an enzyme involved in the biosynthesis and regulation of ethylene (Yang and Hoffman 1984). Furthermore, Dedolph et al. (1966) observed that the auxin sensitivity of clinorotated *Avena* seedlings to applied IAA was increased and a recent study from Li and Wu (1995) reported that the expression of a GUS reporter fused to an auxin-induced promoter sequence (SAUR) was enhanced in *Arabidopsis* seedlings transformed with this construct grown in microgravity or under clinorotation conditions. We believe that clinorotation creates an auxin imbalance within the soybean seedlings and therefore a high concentration of auxin within a specific region of the seedlings would induce ACC synthase and in turn ethylene production. This increase in ethylene would decrease shoot growth and increase root elongation. In the limit of our study, we cannot predict what tissue has the highest concentration of auxin nor if cell sensitivity to auxin is affected by clinorotation.

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